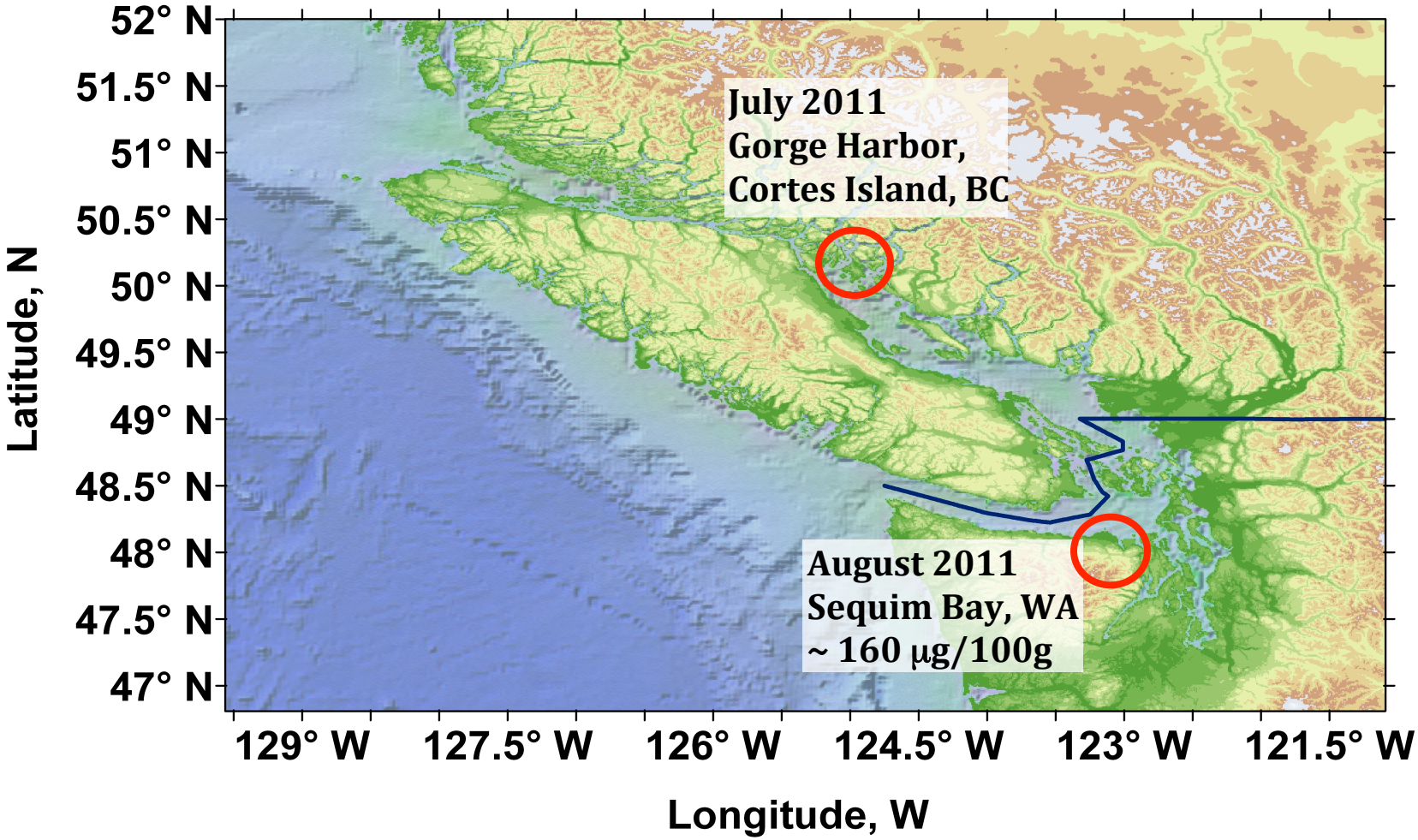
MERHAB: Clear and present danger: monitoring and management of lipophilic shellfish toxins in Washington State

The objectives of the proposed study are to:

1) Identify and spatio-temporally characterize the distribution of phytoplankton species that produce DSP toxins and azaspiracids accumulating in Washington State shellfish,
2) Establish and validate a tiered early warning system for DSP and AZP events, including routine microscopy by SoundToxins/ORHAB partners, and rapid toxin screening in seawater and shellfish,
3) Assist State managers in establishing globally accepted protocols for quantifying lipophilic toxins as part of their biotoxin monitoring program,
4) Inform and educate stakeholders about lipophilic toxin risk and management with the goal of transitioning the project to State funding at the end of 3 years.

2011 Pacific Northwest DSP Outbreaks



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Article

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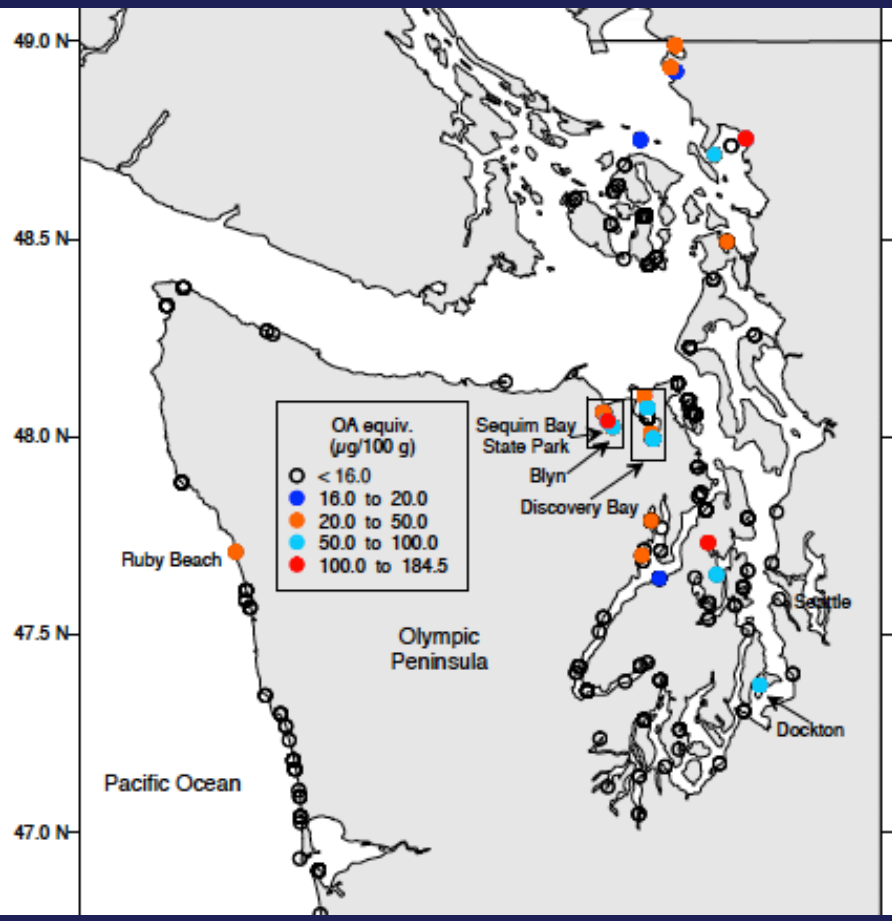
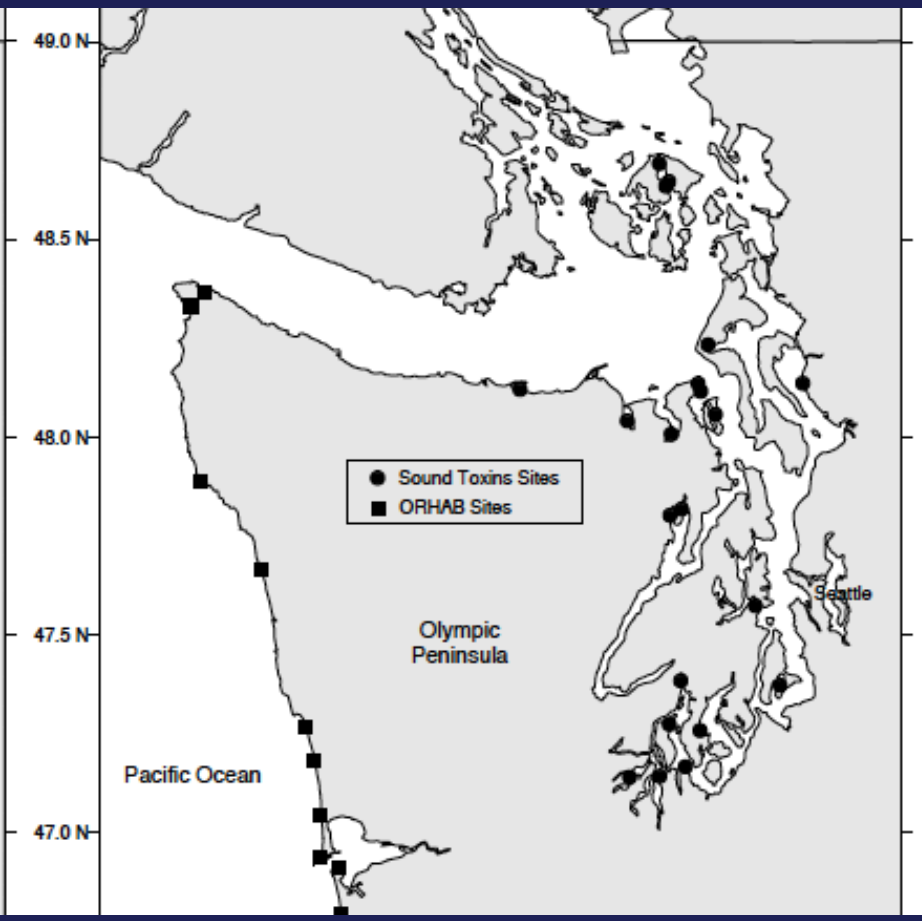
DSP toxins 2012





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Characterization of lipophilic shellfish toxins and associated harmful algal bloom species in Puget Sound (WA) and adjacent coastal waters

Harmful Algae and their Toxins Projects

- Shellfish toxins and associated HAB species in Puget Sound
- Algal toxins in Alaskan marine mammal populations
- Forecasting HABs with weather

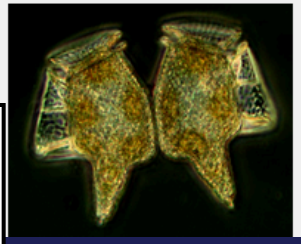
Funding

NOAA National Centers for Coastal Ocean Science (NCCOS) Monitoring and Event Response to Harmful Algal Blooms (MERHAB) program

Partners

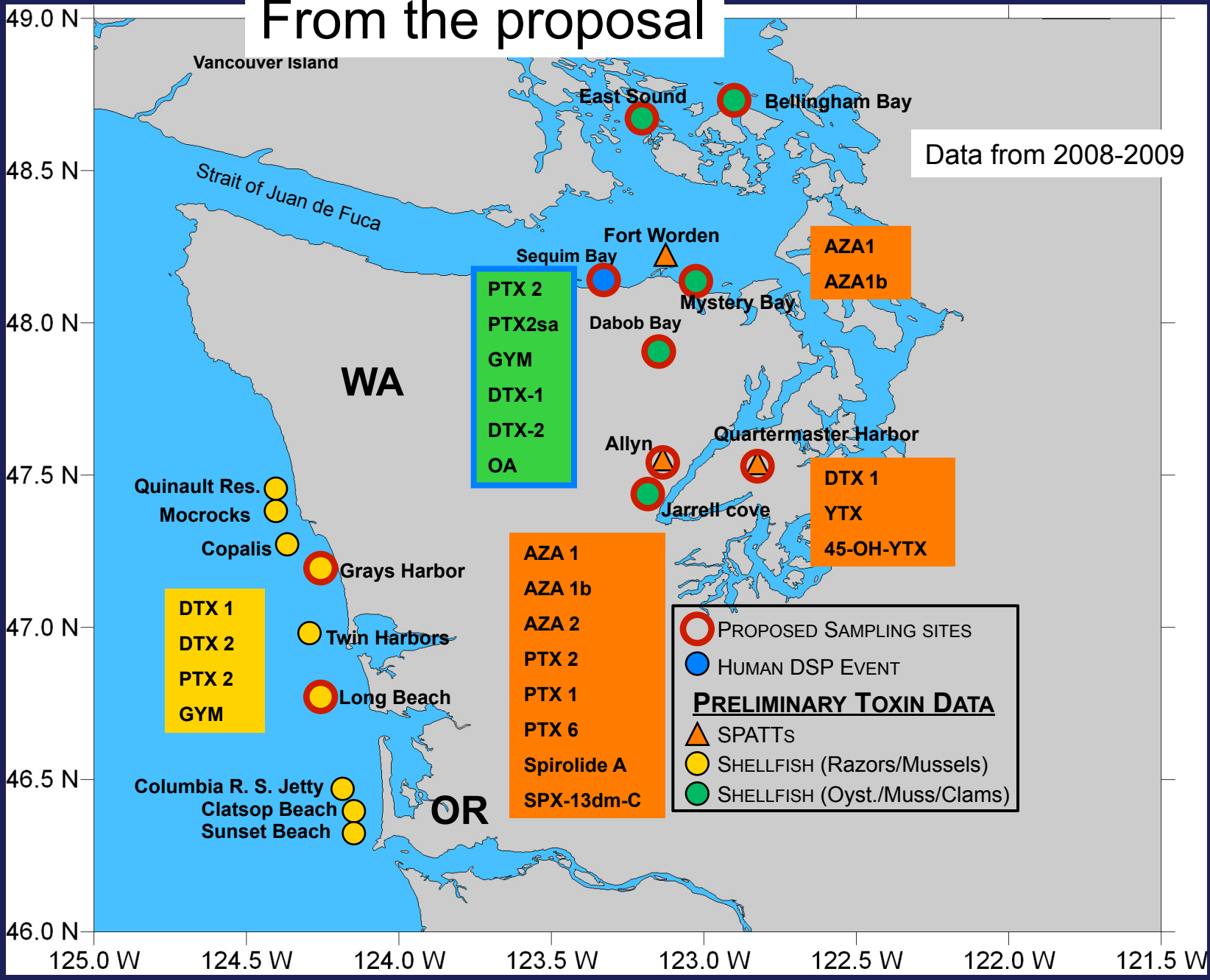
- Jerry Borchert, WA State Department of Health
- Neil Harrington, Jamestown S'Klallam Tribe
- Jonathan Deeds, US Food and Drug Administration (FDA), College Park, MD
- Gregory Doucette, NOAA/NOS/NCCOS/CCEHBR, Marine Biotoxins Program, Charleston, SC
- Urban Tillmann Alfred Wegener Institute, Germany
- Michael Twiner, Wayne State University School of Medicine, MI
- Olympic Region Harmful Algal Bloom (ORHAB) partnership
- SoundToxins partnership

Multimedia



http://www.nwfsc.noaa.gov/research/divisions/efs/microbes/harmful_algae/characterization_lipophilic_shellfish_toxins.cfm

From the proposal



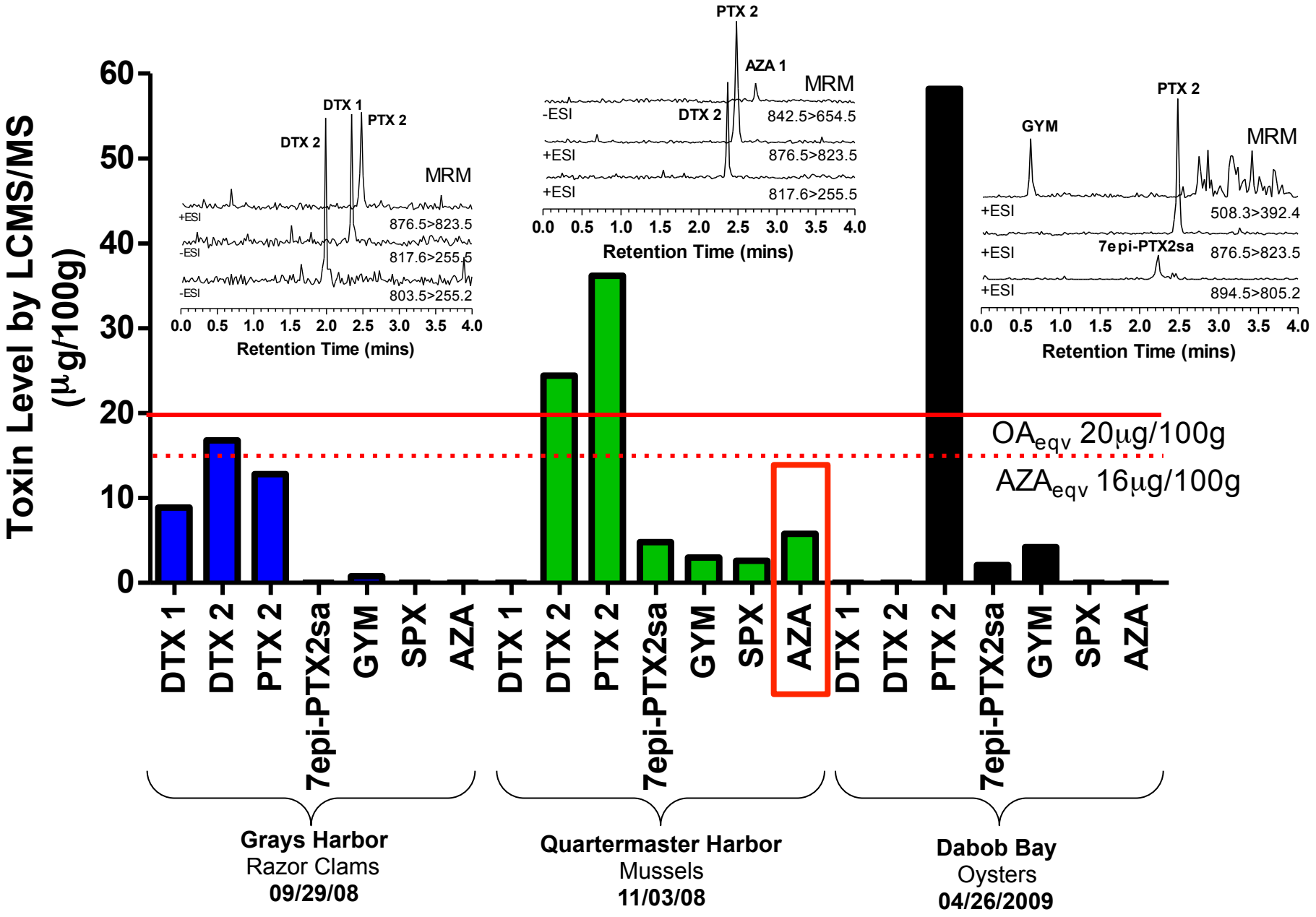

Toxin Level by LCMS/MS
(µg/100g)
60
50
40
30
20
10
0
DTX 1
DTX 2
PTX 2
7epi-PTX2sa
GYM
SPX
AZA
OA$_{eqv}$ 20µg/100g
AZA$_{eqv}$ 16µg/100g
DTX 1
DTX 2
+ESI 876.5>823.5
-ESI 817.6>255.5
-ESI 803.5>255.2
MRM
Retention Time (mins)
PTX 2
AZA 1
DTX 2
-ESI 842.5>654.5
+ESI 876.5>823.5
+ESI 817.6>255.5
MRM
Retention Time (mins)
PTX 2
GYM
+ESI 508.3>392.4
7epi-PTX2sa
+ESI 876.5>823.5
+ESI 894.5>805.2
MRM
Retention Time (mins)
Grays Harbor
Razor Clams
09/29/08
Quartermaster Harbor
Mussels
11/03/08
Dabob Bay
Oysters
04/26/2009

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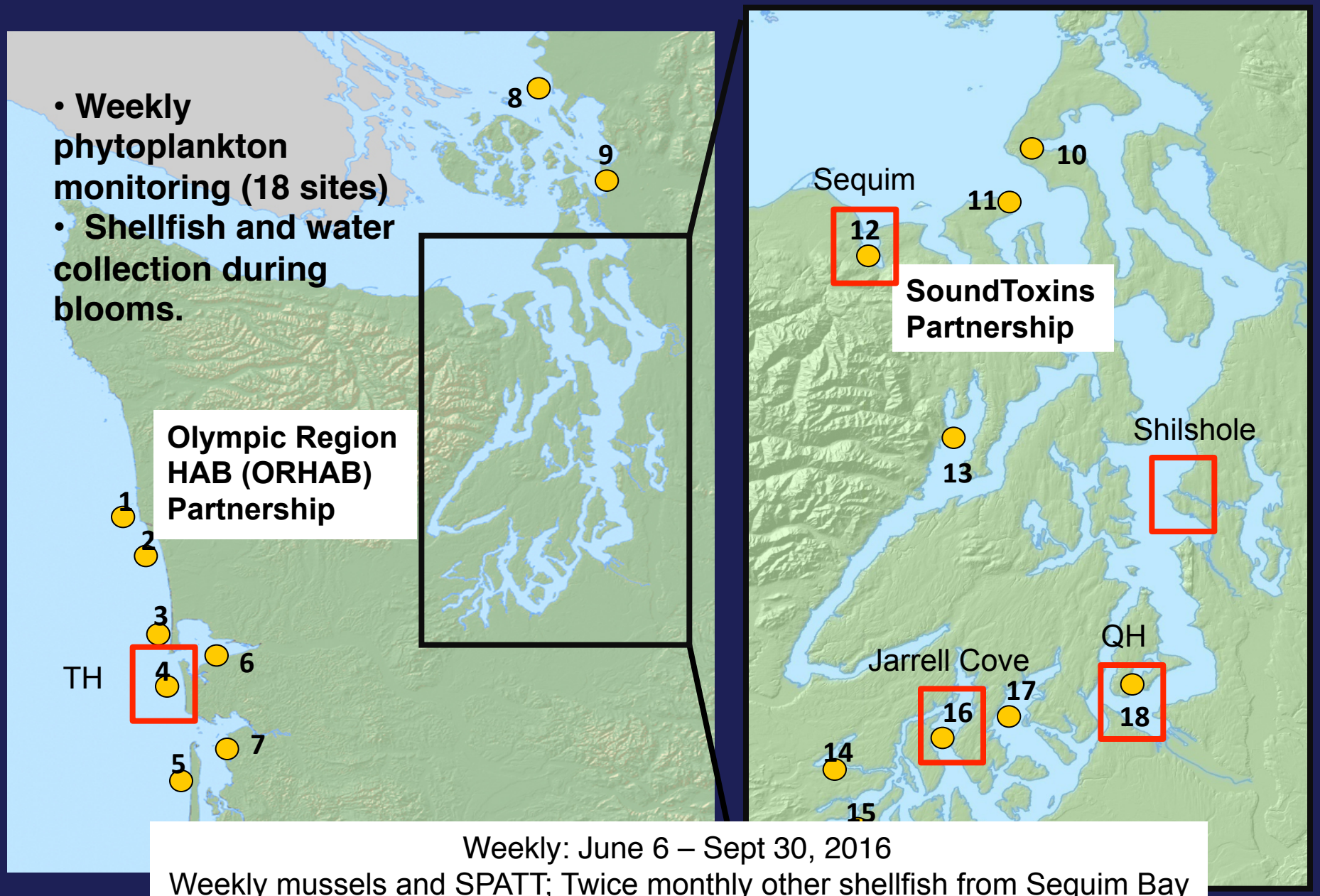
Table 1. Lipophilic toxins [1] measured in Sequim Bay shellfish and phytoplankton during summer 2012.

Date	Sample	Total OA equiv	DTX-1	DTX-3	YTX	PTX2	AZA-2	*Dinophysis* (cells/L)
7/31/12	Blue Mussel	64.68	40.60	24.08	33.78	bd	bd	2000
	Pacific Oyster	3.10	1.25	1.85	1.10	bd	bd	
	Littleneck Clam	7.79	1.28	6.51	bd	1.00	bd	
	Plankton Filter	10.58	7.88	2.70	bd	5.91	0.40	
8/7/12	Blue Mussel	59.30	36.28	23.02	30.93	2.83	bd	8000
	Pacific Oyster	14.27	3.65	10.62	1.51	1.70	bd	
	Littleneck Clam	3.99	0.00	3.99	bd	bd	bd	
	Plankton Filter	38.67	29.74	8.93	bd	33.72	0.46	
8/14/12	Blue Mussel	66.61	42.76	23.85	30.20	bd	bd	1000
	Pacific Oyster	5.67	1.96	3.71	2.88	bd	bd	
	Littleneck Clam	4.21	1.10	3.11	1.43	bd	bd	
	Plankton Filter	11.42	10.13	1.29	bd	8.55	bd	
8/21/12	Blue Mussel	73.48	65.98	7.50	37.26	bd	bd	1000
	Pacific Oyster	8.17	2.77	5.40	3.55	2.77	bd	
	Littleneck Clam	3.19	0.00	3.19	1.62	bd	bd	
	Plankton Filter	15.15	10.31	4.84	3.10	18.65	0.40	
8/28/12	Blue Mussel	51.00	42.52	8.48	34.72	2.51	bd	5000
	Pacific Oyster	26.20	5.59	20.61	4.88	3.49	bd	
	Littleneck Clam	6.69	1.69	5.00	bd	1.85	bd	
	Plankton Filter	30.66	22.62	8.04	6.79	38.36	bd	

[1] OA, DTX-2, AZA-1, AZA-3 were all below the level of detection. Units are µg/100 g for all shellfish and ng/L for plankton filters. [2] na = not analyzed. [3] bd = below the level of detection.

Azadinium project: Shellfish & SPATT sites



Weekly: June 6 – Sept 30, 2016
Weekly mussels and SPATT; Twice monthly other shellfish from Sequim Bay

Timeline from Proposal

Task	Relevant Project Objective(s)	Sept 2014-15				Sept 2015-16				Sept 2016-17			
		Year 1				Year 2				Year 3			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
		Sept-Nov	Dec-Feb	Mar-May	Jun-Aug	Sept-Nov	Dec-Feb	Mar-May	Jun-Aug	Sept-Nov	Dec-Feb	Mar-May	Jun-Aug
Partner training sessions	1,2,3												
Website, Facebook, and Twitter setup and operation	1,2,3,4												
Weekly water samples, phytoplankton collections	1,2												
Weekly shellfish sampling	2												
SPATT deployment	1,2												
Charleston LC/MS analysis	1,2,3												
NWSFC LC/MS analysis	1,2,3												
WDOH LC/MS analysis	1,2,3												
PP2a optimization and deployment (DSP toxins)	2,3												
qPCR optimization using available probes	1,2												
Sequencing and phylogenetic ID	1,2												
Cell isolations and cultures	1,2,3												
qPCR plankton analysis	1,2												
SEM microscopy	2												
Conference meetings	4												
Community Presentations: Dissemination of previous years findings to stakeholders and citizens	3,4												
PI/stakeholder meetings (WA State)	1,2,3,4												
Seek sustainable funding for WDOH toxin program	4												
Modify WDOH toxin program	4												
Publications	1,2,3,4												

Roles & Responsibilities

- **Dr. Vera Trainer** will supervise all sampling and chemical analysis through close collaboration and regular communication with all project partners.
- **Jerry Borchert** (WDOH) will provide advice on shellfish sampling, development of an optimized monitoring program, and community involvement.
- **Dr. Gregory Doucette** will be responsible for initial LC-MS/MS analysis of shellfish and SPATT samples for lipophilic toxins with transfer of this expertise to both the NWFSC and WDOH.
- **Mr. Nicolaus Adams** will work with Dr. Tillman to assess genus- and species-level PCR probes to *Azadinium*
- Ms. Bich-Thuy Eberhart will lead PP2a analysis through close collaboration and regular communication with NWFSC, SoundToxins & ORHAB personnel
- Ms. Catherine Sloan & Gina Ylitalo will lead LC/MS analysis at NWFSC.
- **Dr. Urban Tillmann** (Alfred-Wegener Institute), co-PI, will be involved in the identification of algal species using light and electron microscopy
- **Dr. Jonathan Deeds** (FDA) - evaluating both LC-MS/MS for DSP and AZP and *in-vitro* PP2a for DSP for inclusion in the National Shellfish Sanitation Program.
- **Mr. Neil Harrington** (Jamestown S'Klallam Tribe), co-PI, will sample and analyze samples at Sequim Bay. He will also work to integrate the findings of this project into tribal monitoring and shellfish management programs.